# Synthesis and toxicity evaluation of hydrophobic Ionic Liquids for Volatile Organic Compounds biodegradation in a two-phase partitioning bioreactor

Alfredo Santiago Rodriguez Castillo, Solene Guiheneuf, Rémy Le Guével, Pierre-François Biard, Ludovic Paquin, Abdeltif Amrane, Annabelle Couvert

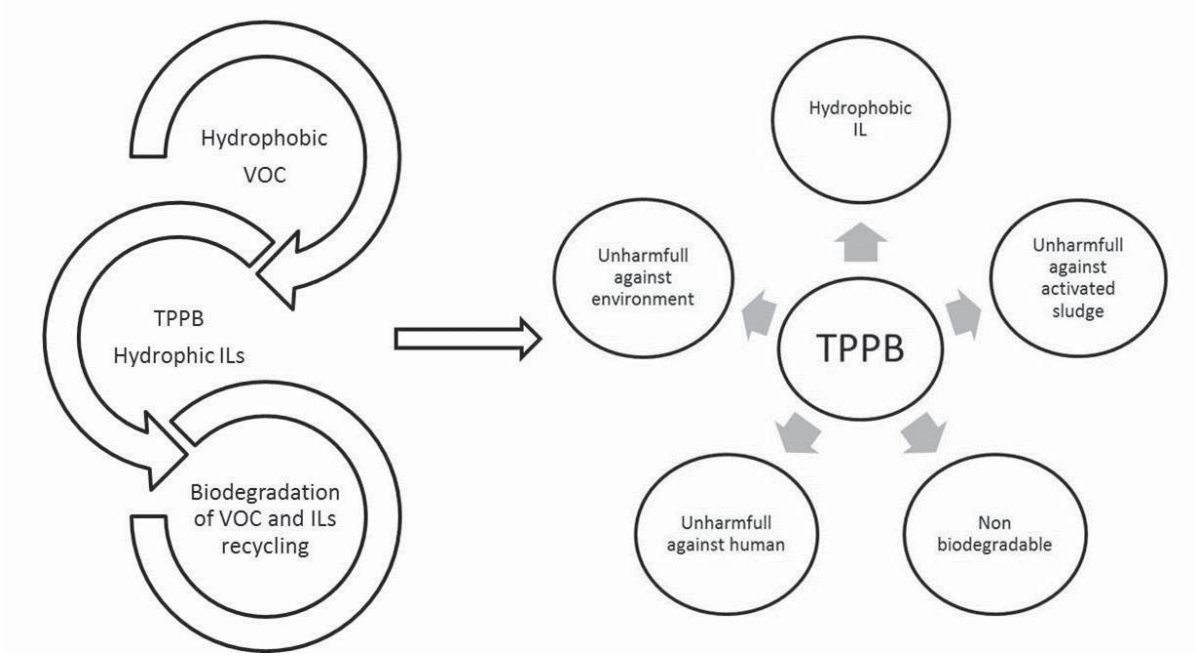
# Synthesis and toxicity evaluation of hydrophobic Ionic Liquids for Volatile Organic Compounds biodegradation in a two-phase partitioning bioreactor.

Alfredo Santiago Rodriguez Castillo[1,2], Solène Guihéneuf[2,3*], Rémy Le Guével[4], Pierre-François Biard[1,2], Ludovic Paquin[2,3], Abdeltif Amrane[1,2], Annabelle Couvert[1,2].

[1]*Ecole Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, 11 Allée de Beaulieu, CS 50837, 35708 Rennes Cedex 7, France.*
[2]*Université européenne de Bretagne.*
[3]*Université de Rennes 1, Sciences Chimiques de Rennes, UMR CNRS 6226, Groupe Ingénierie Chimique & Molécules pour le Vivant (ICMV), Bât. 10A, Campus de Beaulieu, Avenue du Général Leclerc, CS 74205, 35042 Rennes cedex, France.*
[4]*Plate-forme ImPACcell Structure Fédérative de Recherche BIOSIT Université de Rennes 1, Bat 8 Campus de Villejean 2 Avenue du Pr. Leon Bernard CS34317 35043 Rennes cedex, France.*

*Corresponding author e-mail address : solene.guiheneuf@wanadoo.fr

Graphical abstract



Highlights

- Description of a VOC depollution system suitable with industrial processes, TPPB.
- Novel association of TPPB and hydrophobic ionic liquids.
- Synthesis of several hydrophobic ionic liquids designed to fit desired properties.
- Toxicity evaluation of these ILs towards cells, animals and bacteria.

Abstract

Synthesis of several hydrophobic ionic liquids (ILs), which might be selected as good candidates for degradation of hydrophobic volatile organic compounds in a two-phase partitioning bioreactor (TPPB), were carried out. Several bioassays were also realized, such as toxicity evaluation on activated sludge and *Zebrafish*, cytotoxicity, fluoride release in aqueous phase and biodegradability in order to verify their possible effects in case of discharge in the aquatic environment and/or human contact during industrial manipulation. The synthesized compounds consist of alkylimidazoliums, functionalized imidazoliums, isoqinoliniums, triazoliums, sulfoniums, pyrrolidiniums and morpholiniums and various counter-ions such as: $PF_6^-$, $NTf_2^-$ and $NfO^-$. Toxicity evaluation on activated sludge of each compound (5% v/v of IL) was assessed by using a glucose uptake inhibition test. Toxicity against *Zebrafish* and cytotoxicity were evaluated by the ImPACCell platform of Rennes (France). Fluoride release in water was estimated by regular measurements using ion chromatography equipment. IL biodegradability was determined by measuring $BOD_{28}$ of aqueous samples (compound concentration,1 mM). All ILs tested were not biodegradable; while some of them were toxic toward activated sludge. Isoquinolinium ILs were toxic to human cancerous cell lines. Nevertheless no toxicity was found against *zebrafish Danio rerio*. Only one IL released fluoride after long-time agitation.

Keywords: Ionic liquids

Biodegradation

Toxicity

Synthesis

# 1. Introduction

## *VOCs - Volatile Organic Compounds[1] - absorption : "a green challenge"*

Air quality, particularly VOC emissions and their consequences, has been preoccupying scientists for 40 years, considering that these compounds can be toxic for human beings, and often odorous. This emerging pollution issue, correlated with industry growth [2] or with domestic uses [3], is a public health question and since the beginning of the nineties, several methods have been described in the literature to catch and degrade VOCs [4,5,6]. One of these methods is absorption, either physical or chemical. For a long time, most of the related reports have been concerning absorption of VOCs in aqueous phases and targeted hydrophilic compounds. Unfortunately, problematic VOCs are often hydrophobic (benzene, toluene…). Thus, hydrophobic VOC emission control are based on membrane use strategies [2,7], adsorption on activated carbon [8], absorption by polymers [9], thermal or catalytic oxidations [10], or biofiltration [11], rarely on absorption. A promising strategy consists in the use of TPPBs (Two-Phase Partitioning Bioreactors) [12,13]. Their principle consists in absorbing targeted compounds in a non-aqueous phase liquid (NAPL), exhibiting high affinity for pollutants from a gaseous flow, and coexisting with an aqueous phase containing microorganisms able to degrade the VOCs absorbed [13,14].

In previous works, VOC treatment in a TPPB using silicone oil PDMS as NAPL was described [15,16]. Another kind of solvent which can be used and which is not largely studied is ionic liquids (ILs). ILs are safe (low vapor pressure, weakly flammable …) and easily tunable [17,18]; they can be designed to fit the criteria required by the TPPB process [19]. This ability of ILs to be modified to correspond to specific properties (viscosity, hydrophobicity, biodegradability …) is a serious advantage [20,21,22,23,17,24,25]. Nowadays, TPPBS essays have been done for toluene in a TPPB of 16L with very interesting results. High degradation rates and no gas stripping with the selected aeration rate were observed. With a 5% IL-water ratio, the amount of toluene that can be eliminated compared to the same reactor without IL was 6 times higher without toluene losses by gas stripping.

Biodegradation kinetics in Erlenmeyer flask have been done for toluene and dichloromethane. Some really interesting results have been found.

## *Ionic Liquids: good candidates for TPPB applications*

ILs are salts composed by an organic cation associated with an inert anion and melting under 100°C [26,27,28]. They are generally considered as green solvents, since they have low vapor pressure and can often be recycled after the process [29,30]. ILs have sometimes been described for whole-cell applications [31,32] and hydrophobic ILs are known to be good absorbents for hydrophobic organic compounds [33,34]. They exhibit many advantages for bioreactor applications according to their tunable properties depending on their structure and their safety

[35]; the assessment of ILs performances as NAPL for TPPB degradation of VOCs was therefore examined in this work. We previously reported a toxicity and biodegradability study based on four ILs ([Bmim][$Pf_6$], [Bmim][$NTf_2$], Aliquat and [PEGmim][$Pf_6$]) and showed that only Aliquat is toxic towards activated sludge [36] and [PEGmim][$Pf_6$] is soluble in water; these two criteria excluded their use for this application, while all these ILs are non-biodegradable [13].

Partition coefficient are more interesting between IL and hydrophobic VOC than those already found in literature (even better than other solvents studied to eliminate VOC) (Table 1):

This means that larger amounts of VOC can be treated with the same amount of IL. Therefore, the process size can be adjusted to the amount of VOC to be treated. Nowadays, IL-water ratio has not been optimized yet. After optimization, the process size could be even smaller than expected with the actual value of 5%.

ILs Separation and recycling process will be also easier given the differences of viscosity (> water) and density (30 – 50 % > water) of IL compared to water.*A priori*, a simple process of decantation is enough to recover more than 90% of IL.

The main obstacle is the toxicological profile of ILs as they have to be harmless toward microorganisms present in the bioreactor but also toward the environment (in case of discharge in the aquatic environment) and human (industrial staff manipulating ILs). This paper reports toxicity study of ILs selected as good candidates for degradation of hydrophobic VOCs. Syntheses of the considered ILs were described; toxicity evaluation on activated sludge and zebrafish, cytotoxicity, fluoride release in aqueous phase and biodegradability were examined.

It is also relevant to signal that the current cost of ionic liquids is high. However, in this work, ILs have been synthesized in the lab to reduce costs. In addition, those requiring too steps for their synthesis have been avoided. Moreover, this work is a prospective study, which is upstream of a possible industrial application. It should also be remembered that costs synthesis decrease year-by-year…. Therefore and if an ionic liquid shows a real potential for the proposed application, a possible application can be expected in a more or less near future. We expect that in the future, the high investment cost related to IL supplying will be covered by a significant functioning cost decreasing, related to the high efficiency of IL for hydrophobic VOC absorption.

## 2. Experimental procedures

### *Synthesis of ILs*

In order to fit the specifications of the NAPL (hydrophobicity, viscosity, toxicity, density …), the related literature was browsed to select IL structures. The hydrophobicity criterion made us choose classical anions (e.g. PF6 and NTf2) but we narrow down the choice by excluding expensive anions (e.g. FAP).We also performed a bibliographic preparation, exploring some properties as viscosity, toxicity or melting point, that are primordial properties to considerate for TPPB application. We excluded ILs solid at RT, partially soluble in water or expensive to synthetize. Easy synthetic access and low cost reagents (except for some structure as perfluorinated triazolium selected to evaluate the influence of fluorinated groups) were also a criterion. We also tried to diversify the scaffolds whereas a lot of studies are focused on one or two ILs families (mostly imidazoliums). In a first step, 23 ILs were synthesized according to classical conditions involving an alkylation reaction with the appropriate halide and an anion exchange. Several cationic scaffolds were explored (imidazoliums, isoquinoliniums, pyrolidinims, morpholiums, triazolium and sulfoniums) including functionalized or non-functionalized alkyl side chains and associated with various anions ($PF_6^-$, $NTf_2^-$ and $NfO^-$): [nPrMim][$NTf_2$], [BMim][$PF_6$], [BMim][$NTf_2$] [40], [iPtMim][$PF_6$], [iPtMim][$NTf_2$], [ButenylMim][$PF_6$] [41,42], [ButenylMim][NfO], [ButenylMim][$NTf_2$], [MeOEmim][$NTf_2$] [43], [MeOEMim][NfO], [EtOEMim][$NTf_2$], [$MeOC_2OC_2Emim$][$NTf_2$] [44,45], [$CNC_3Mim$][$NTf_2$] [46], [OctIq][$NTf_2$], [$C_{10}Iq$][$NTf_2$] [47] and [MeOEIq][$NTf_2$], [EtOEIq][$NTf_2$], [BMPyrr][$NTf_2$], [MeOEMMorph][$NTf_2$], [EtOEMMorph][$NTf_2$], [BMTriaz][$NTf_2$], [$CF_2CFEBTriaz$][$NTf_2$] [48], [$AllylEt_2S$][$NTf_2$] [49]. Besides, in order to obtain high quantities of the final selected IL, a CFMR (Continuous Flow Microwave Reactor) study was initiated to increase the synthesis capacity.

### *Toxicity against activated sludge*

The ionic liquid toxicity was determined by glucose uptake inhibition according to Baumann experiments [50]. Tests were conducted in 120 mL glass bottles containing 20 mL of biological media. The bottles were closed with PTFE/silicone septa (Sigma-Aldrich, USA) and sealed with 20 mm crimp seals. Each assay was repeated twice while a set of bottles was supplied with different solutions in order to ensure growth and viability of the microorganisms. The bottles were maintained under constant agitation (300 rpm) at 25°C for 48 h using the Innova 40 Incubator Shaker series (Eppendorf, USA). Samples (200 µL aqueous solution) were taken each 2 h during working days.

IL toxicity test consists in mixing activated sludge inoculums of 0.5 $g_{DCW}.L^{-1}$ and Trinci's mineral salt medium [51] to glucose at a final concentration of 2 $g.L^{-1}$. Then, pH must be

adjusted at 7.0±0.2. The considered IL concentration was 5% v/v.
To avoid any additional nutrient other than those contained in the culture medium, the activated sludge must be washed as follows: 200 mL of sludge were centrifuged at 3500 rpm for 10 min and the supernatant was removed. The pellet was resuspended with ultrapure water and after vigorous agitation, the volume was centrifuged again. The supernatant was removed and the pellet was washed again (with ultra pure water and after vigorous agitation) and was filtered (sieving diameter = 0.2 mm). The filtrate was then centrifuged and the supernatant was removed. The pellet was finally resuspended in ultra pure water and then the dry cell weight (DCW) was measured (≈ 4 $g.L^{-1}$). The glucose concentration was measured through spectrophotometric measurements at 420 nm (Helios ϒ spectrophotometer, Thermo Spectronic, France) of aqueous samples after reaction with glucose oxydase/peroxidase [52] enzymes in the presence of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) [53].

### *Fluoride release analysis in water*

The IL was introduced in ultrapure water (25% vol.) and magnetically stirred. Fluoride was quantified regularly by ionic chromatography (881 Compact IC pro – Anion from Metrohm). The samples were prepared as follows: after stopping the stirring, 10 µL of the aqueous layer were diluted in 1 mL of analysis solvent (ultrapure water / acetonitrile HPLC grade 75:25). The eluent was composed of $NaHCO_3$ (4 mM) and $Na_2CO_3$ (1 mM) in ultrapure water / acetonitrile HPLC grade 75:25 and the flow rate was set up to 1 $mL.min^{-1}$. The column was a Metrosep A supp 4 from Metrohm with the suitable precolumn from Metrohm and the oven temperature was 45°C.

### *Cytotoxicity*

ILs cytotoxicity was evaluated by the ImPACCell platform in Rennes using the following cell lines: Caco2 and HCT-116 (human colon tumor), Huh-7D12 (human hepatoma), MDA-MB (human mammary carcinoma), PC3 (human prostate tumor), NCI-H727 (human lung tumor), Fibro (human skin fibroblast) and HaCaT (skin cells).

Taxol, Roscovitine and Doxorubicine were used as references to compare cytotoxicity of the tested products. Their $IC_{50}$ are given above in µM (Table 2).

A first experiment with 25 µM samples showed that most of the ILs are not cytotoxic against these cell lines. For the compound exhibiting relevant inhibition percentage, a second test was performed to determine the $IC_{50}$ (µM).

### *Toxicity against zebrafish Danio rerio*

The experiments were performed using a Thermo Scientific Cellomics ® Zebratox kit from the Thermo Scientific company (Pittsburg, Pennsylvania, USA) in collaboration with the ImPACCell plateforme (Rennes, France). The Zebrafish toxicological test (Zebratox) was realized as follows: the 24h post fertilization (PF) embryos were arrayed by hand with one embryo per well in a black walled 96-well microplates for fluorescence assays (PerkinElmer).The embryos were treated after hatching at 48 hour PF. The 20mM DMSO stock compounds were diluted in fish water and added to the wells at final concentrations of 25, 50 and 100 µM. The negative control (0.25% DMSO) was added in each replicate plate. The experiments were realized in triplicate in three independent plates.

The embryos were incubated for 72 hours at 28°C and were imaged, after anesthesia by incubation at 4° C for 30 minutes, on an ArrayScan VTI HCS Reader (Thermo Scientific) with a Brightfield module at 1.25x magnification and a 0.63x coupler. The Zebratox BioApplication was used for automated analysis of zebrafish embryos morphological parameters.
The wild type zebrafish fertilized eggs were provided by the INRA LPGP zebrafish facilities (Rennes, France).

### *IL Biodegradability: $BOD_{28}$ (Biological Oxygen Demand) tests*

Prior to biodegradability tests, the chemical oxygen demand (COD) was determined. The COD represents the amount of oxygen required to oxidize organic and inorganic oxidizable compounds present in water according to the norm ISO 15705. The result is expressed in milligrams of oxygen per liter of solution ($mgO_2.L^{-1}$).

All synthesized ILs were liquid at room temperature (RTILs) and hydrophobic, but we observed that they were soluble in water up to 1 mM. Hence, COD and BOD values for ILs were determined at that concentration.

COD was measured using Macherey-Nagel kits which contain Nanocolor tubes test. The test consists in a silver-catalyzed oxidation with potassium dichromate/sulfuric acid at 148°C during a two hour period for the range of 100 – 1500 $mgO_2.L^{-1}$. For each test, 2 mL of ILs solution is required. COD is determined by a photometric determination based on the chromate concentration decreasing (wavelength 620 nm or using the Nanocolor Photometers 500D).

The biological oxygen demand tests ($BOD_{28}$) were then performed according to Darracq et al. experiments [37,54]. $BOD_{28}$ tests were based on the amount of oxygen required by an activated sludge to metabolize the IL during 28 days. The IL biodegradation is expressed as a

percentage of oxygen depletion relative to the theoretical chemical oxygen demand (COD):

$$\% \text{ Biodegradation} = [\ (BOD_{28} - BOD_{28}^{EB}) / COD\ ] \times 100, \qquad (\text{Eq. 1})$$

where $BOD_{28}^{EB}$ is the $BOD_{28}$ value of the endogenous oxygen uptake controls. Additional controls with glutamic acid and glucose as carbon sources were performed to assess the microbial activity. Compounds with biodegradation percentages higher than 60% are considered as readily biodegradable [55]. Experiments were made in duplicate.

## 3. Results and Discussion

### *Toxicity against activated sludge*

This criterion is crucial for IL implementation in a TPPB and has therefore to be considered first.

To assess glucose uptake, two parameters were considered in agreement with a previous study[36], the first part of the glucose time-course, the lag time which characterize activated sludge acclimation to the IL, and the second part of the glucose time-course, when the cells recover a metabolic activity, namely the time needed for total glucose consumption which is linked to the glucose consumption rate.

Toxicity tests showed that from the 23 ILs investigated, three of them ([BMtriaz][$NTf_2$], [Butenylmim][NfO] partially and [MeOEmim][NfO] completely) inhibited the microbial glucose uptake at a concentration of 5%. For all ILs, an acclimation time (lag time) was observed, varying between 16 h and 28 h. Then, glucose was consumed more readily in the presence of some ILs if compared to others (Table 3). If the glucose concentration was greater than 0.10 $g.L^{-1}$ ($A/A_0 = 0.05$) after 48 h, the IL was considered as toxic. These results suggest that microorganisms are able to recover totally or partially their metabolic activity after being exposed to the considered ILs, and hence from these results the global toxicity of the ILs is, as expected, linked to each different association cation + alkyl chain / anion. From this, the cells must be acclimated to the IL to recover significant metabolic activity, which finally means a supplementary cost to the target process. Therefore, a previous acclimation step of the microbial inoculum must be assessed in order to avoid or minimize the lag time in the process.

Figures 1.a to 1.f summarize the glucose consumption kinetics for the studied ILs. Results for [$NTf_2$] ILs are represented in figures 1.a to 1.d, while [$PF_6$] and [NfO] anions are represented in figures 1.e and 1.f respectively. The results confirmed that the alkyl chain length and the number of carbon atoms influence significantly IL toxicity. Indeed, for two ILs with the same core and anion, e,g. the couple [OctIq][$NTf_2$]/[$C_{10}$Iq][$NTf_2$] or

[nPrmim][$NTf_2$]/[Bmim][$NTf_2$], the lag time reduced when the alkyl chain length increased which is in contradiction with the related literature [31,56,57]. The variation of the acclimation time for ILs with $NTf_2$ anion follows the order (Table 3):

$$[Triaz] > [Morph] > [S] > [Pyrr] \geq [Im] > [Iq]$$

Nevertheless, the time required for glucose consumption seems to not vary a lot. ILs with longer alkyl chain should however be subsequently tested in order to complete the study regarding the effect of the alkyl chain on microbial toxicity. Besides, it was found that the addition of a double bound or a methyl group to the alkyl chain has a negative impact on the acclimation time. Moreover, this impact is also linked to the anion, which can induce toxicity as found for instance for [Butenylmim][NfO]. In the case of the $NTf_2$ anion, the time needed for glucose consumption decreased in the following order (Table 3):

$$[Pyrr] = [Triaz] > [Morph] \geq [S] = [Im] > [Iq]$$

Regarding the cation effect in IL toxicity, results show that lag time varies as follows:

$$[NfO] > [PF_6] > [NTf_2]$$

While the time needed for glucose consumption follows:

$$[PF_6] > [NfO] > [NTf_2]$$

For both parameters, the [$NTf_2$] anion appears to be the less toxic. From this, ILs containing the [$NTf_2$] anion with different cation + alkyl chain configurations appeared to be the most relevant to be tested for implementation in the proposed application.

Ether / hydroxyl-functionalized ILs are cation-modified ILs which has been reported in the literature to be less toxic than their non-functionalized analogue [58]. Indeed, even though they seem to be more water soluble than their non-functionalized analogue, they diffuse less rapidly in water, and therefore they interact less with the microorganisms present in the system. In our case, all studied ILs are immiscible in water (concentration in solution up to 1mM), and thus toxicity towards microorganisms must be correlated to the presence of two liquid phases in the system. The obtained results show that ether-functionalized ILs are not toxic as expected, however the acclimation time and the glucose consumption time vary according to the cation type and the alkyl chain size for imidazolium ILs (Figure 1.c). The –O position in the alkyl chain seems to have a clear impact on the time needed for glucose consumption; while the acclimation time appears to be similar to those of their non-functionalized analogue (e,g. [MeOEmim][$NTf_2$]/[Bmim][$NTf_2$]). The acclimation time increased with the alkyl chain length and consequently with the presence of several ether groups in the alkyl chain. Variation of acclimation time for hydroxyl-functionalized ILs follows the order (Table 3):

$$[MeOC_2OC_2Mim][NTf_2] > [EtOEmim][NTf_2] > [MeOEmim][NTf_2].$$

Regarding glucose consumption time, it decreased when alkyl chain length increased (e,g. [EtOEmim][$NTf_2$] < [MeOEmim][$NTf_2$]). Contrarily, for isoquinoliniums ILs (Fig 1.b), ether-functionalized ILs cannot be compared with their non-functionalized analogue because they are solids at room temperature, and hence they are not eligible for implementation in the considered application. Nevertheless, results suggest that the presence of oxygen in the side alkyl chain does not affect microorganisms' behavior, due to the cation size which has a higher effect towards microorganisms than the side alkyl chain. Consequently, acclimation time and glucose consumption remained unchanged in the presence of whether [MeOEIq][$NTf_2$] or [EtOEIq][$NTf_2$].

For morpholinyniums ILs (Fig 1.c), the –O position in the alkyl chain modifies directly their kinetics, both the times needed for acclimation and glucose consumption decreased when the alkyl chain is longer [EtOEMMorph][$NTf_2$] < [MeOEMMorph][$NTf_2$].

*Toxicity against Zebrafish Danio rerio*

Zebrafish is an aquatic organism often used to evaluate ILs impact on the environment. Pretti et al.[59,60] and Du et al.[61,62,63] published studies reporting acute toxicity of ILs and the mechanisms involved. Results showed that alkylimidazoliums and pyrrolidiniums ([BMim][$PF_6$], [BMim][$NTf_2$] and [BMPyrr][$NTf_2$]) exhibit $LC_{50}$ (median lethal concentration) higher than 100 $mg.L^{-1}$ after 96 h exposure. Histological studies demonstrated that ILs exposure induced a reduction of general activity and erratic swimming [59]. It is commonly admitted that IL toxicity to aquatic organisms is mostly directed by the cationic moiety and especially by the side chain length [60]. The ILs concerned by our work can be considered as practically harmless to *Danio rerio* (median effective concentration $EC_{50}$ range from 100 to 1000 $mg.L^{-1}$). In order to establish the impact of ILs on zebrafish organism, Du et al. (2012) considered the effect of the hydrophobic [OctMim][$PF_6$] after 28 days exposure. As [OctMim][$PF_6$] inhibited antioxidant enzymes activities, the authors observed an accumulation of ROS (reactive oxygen species) and DNA damages (mutations) that could explain the toxicity of ILs towards several microorganisms [62].

Toxicity studies were performed in collaboration with the ImPACCell plateforme (Rennes, France) to evaluate both influence on both egg hatching and further development. The evaluation was performed using several IL concentrations: 10, 25, 50 and 100µM (Figure 2). DMSO (0.25µM) and Doxorubicine (0.5µM) were used as controls. First, it was observed that the hatching was not altered by the presence of the tested ILs, no matter the concentration. Concerning the fish's development, various parameters were considered, such as the fish area (indication for growth retardation), dynamic behavior, morphology (fish shape, head-tail distance and fish curvature) and mortality. Up to 100 µM IL, no effect was noticed on the fish's development after 72h. Standard growth rhythms were measured, the swimming behavior was not modified; morphological parameters fitted standard values and no mortality

was detected.

### *Fluoride release in water*

Another parameter to assess is the hydrolysis of some anions (e.g. hexafluorophosphate) which has been described as influencing the toxicity of ILs on several targets, especially microorganisms [17,23,64,65,66]. In a previous work, we showed that in the case of [Bmim][$PF_6$] and [Bmim][$NTf_2$], fluoride release was not high enough to induce a toxic effect [36]. An evaluation of fluoride toxicity towards activated sludge established that aerobic glucose-degrading bacteria could support high concentrations of fluoride reaching 500 $mg.L^{-1}$ [67]. Fluoride release in water with an IL ratio of 25% vol. was monitored and the results are presented in Table 4.

These results show that only few ILs generate fluoride production in relevant quantities. [iBuMim][$PF_6$] inducing low fluoride concentrations in the aqueous phase and in principle, this concentration would not induce toxic effect according to previous reports in the literature [36,67]. However, in the case of [$CF_2$CFEBTriaz][$NTf_2$], fluoride amounts were very high (from 200 $mg.L^{-1}$ to 1$g.L^{-1}$) and can cause toxic effects towards bacteria and microorganisms. Hence this IL must be removed from the project even if no toxic effects have been observed towards bacteria and zebrafish. According to the literature, more significant fluoride release was expected, especially concerning hexafluorophosphate and bis(trifluorosulfonyl)imide based ILs. However, from these results it appears that the side chain could have a preponderant role. For example, the fluorinated side chain of the [$CF_2$CFEBTriaz][$NTf_2$] seems to induce intense fluoride release in the aqueous phase whereas the bis(trifluorosulfonyl)imide based triazolium does not.

### *Cytotoxicity*

For an industrial or a semi-industrial application, it is necessary to establish a complete toxicity profile, including toxicity towards human, even if the IL will not be in direct contact with operators. Several exhaustive reports were published in 2010 and 2011 and summarize all known studies concerning cytotoxicity of ILs towards mammalian cell lines (HeLa, CaCo-2, IPC-81, HT-29, C6, MCF-7, NCI60, V79). They confirmed the interdependence between lipophilicity, structure, concentration and cytotoxicity [65,68]. About 230 ILs with various structures were evaluated on IPC-81 (mammalian cell lines derived from a model of acute myelogenic leukemia) and a QSAR/QSTR (Quantitative Structure Activity-Property

Relationships) profile was established, confirming the classical tendencies (chain length, hydrophobic anions…) [69].

For this purpose, the synthesized ILs were submitted to a cytotoxicity evaluation. The first evaluation at a 25 µM concentration showed that only isoquinoliniums could be considered as potentially cytotoxic against the involved cell lines (Table 5). A second evaluation led to $IC_{50}$ measurements in a micromolar range of concentration, from 1 to 31 µM (Table 5), which indicates that these ILs are cytotoxic towards the considered cell lines.

It is interesting to note that the introduction of an alkoxy group on the isoquinolinium scaffold decreased the cytotoxicity in agreement with the literature [70]. Indeed, the [MeOEIq][$NTf_2$] did not inhibit cell proliferation whereas [OctIq][$NTf_2$] did. Moreover, the alkyl side chain effect between $C_6$, $C_8$ and $C_{10}$ was not obvious, while the $C_4$ homolog was not cytotoxic (no inhibition observed at 25 µM) (Table 5).

### *Biodegradability*

Coleman et al. proposed an exhaustive summary of the biodegradability studies reported in the literature [22]. Our previous results were in agreement with the literature [31,55] and the previous evaluated ILs were shown to be non-biodegradable in contact with activated sludge. Indeed, with an imidazolium-based cation, irrespective of the considered anion, no biodegradation was observed. Similarly, Stolte et al. [54] reported that activated sludge is not able, even after 31 days, to assimilate [Bmim] cations.

Functional groups not easily biodegradable (nitriles, ethers …) were selected, while esters or amides were excluded to avoid enzymatic cleavage.

The $BOD_{28}$ tests showed that all ILs are not biodegradable; negligible biodegradation percentages were obtained for all ILs (Table 6). In addition, for ILs, the $BOD_{28}$ values were notably lower than those obtained for the endogenous oxygen uptake controls. Even if the difference was small, results suggest that metabolic activity of microorganisms was affected by the presence of IL which was in agreement with the results of the glucose uptake inhibition tests where an acclimation time was observed before the occurrence of glucose consumption. Therefore, $BOD_{28}$ tests indicate that none of the ILs tested were readily biodegradable, even at a concentration of 1 mM.

The results herein obtained from $BOD_{28}$ are in agreement with the literature. Different imidazolium ILs were tested with different anions such as [Br], [Cl], [$NTf_2$] and [$BF_4$] using activated sludge to assess IL biodegradability and concluded that none of the tested

combination was biodegradable [71,72]. Moreover, no biodegradability of the [Bmim] cation in 31 days was reported [73,74]. Nevertheless, partial biodegradation was observed for imidazolium-based cations for ILs with long alkyl chains (e.g. $C_8$ chains instead of $C_4$ as in [Bmim]). As a matter of fact, biodegradation of the alkyl chain ceased when the chain length reached two carbon atoms and the aromatic ring remained intact [75]. Concerning our results, the absence of biodegradability of hydroxyl-functionalized ILs and non-functionalized ILs with long alkyl chains (such as [$MeOC_2OC_2Mim$][$NTf_2$] and [$C_{10}Iq$][$NTf_2$] respectively), while the contrary was expected, suggests an important anion effect towards ILs biodegradability.

The present work assessed the toxicity of 23 hydrophobic ILs towards activated sludge and *Zebrafish*, cytotoxicity, fluoride release in aqueous phase and biodegradability of ILs. The results show that only 3 ILs inhibited glucose consumption in the case of activated sludge, but for microorganism such as *Zebrafish*, no inhibition of fish's development was found. Only isoquinoliniums could be considered as potentially cytotoxic against the involved cell lines; however activated sludge seemed to be less stressed by the presence of isoquinolinium ILs than in the presence of any other IL. Moreover, none IL was found to be biodegradable. Results also show that the side chain could have a preponderant role in fluoride release, thus explaining why the fluorinated side chain of the [$CF_2CFEBTriaz$][$NTf_2$] induces intense fluoride release in the aqueous phase whereas the bis(trifluorosulfonyl)imide based triazolium does not. Therefore, these results show that while most of these compounds are classified as "green solvents" given their physico-chemical properties, their toxicity vary from one microorganism to another and that their future application for air treatment by TPPB should be feasible; nevertheless, precautions should be taken during handling.

## 4. Acknowledgements

The authors want to thank the ANR for the founding support (ANR 12 BLANC 007 01) and the ImPACCell platform for the cytotoxicities of ILs (Rémy Le Guével and Myriam Ravache). We also want to thank the wastewater treatment plant of Rennes, France for providing the activated sludge.

## 5. References


(1) Host, S.; Gremy, I. *Les composés organiques volatils ( COV )*; 2007.

(2) Peinemann, K. V.; Ohlrogge, K.; Wind, J. Industrial applications of membranes to control VOC emissions. *Stud. Environ. Sci.* **1994**, *61*, 375–385.

(3) Bourgeois, P.-A.; Puzenat, E.; Peruchon, L.; Simonet, F.; Chevalier, D.; Deflin, E.; Brochier, C.; Guillard, C. Characterization of a new photocatalytic textile for formaldehyde removal from indoor air. *Appl. Catal. B Environ.* **2012**, *128*, 171–178 DOI: 10.1016/j.apcatb.2012.03.033.

(4) Ruddy, E. N.; Caroll, L. A. Select the best VOC control strategy. *Chem. Eng. Prog.* **1993**, *89* (7), 28–35.

(5) Revah, S.; Morgan-Sagastume, J. M. Methods of Odor and VOC Control. In *Biotechnology for Odor and Air Pollution Control*; 2005; pp 29–63.

(6) Schlegelmilch, M.; Streese, J.; Stegmann, R. Odour management and treatment technologies: an overview. *Waste Manag.* **2005**, *25* (9), 928–939 DOI: 10.1016/j.wasman.2005.07.006.

(7) Majumdar, S.; Bhaumik, D.; Sirkar, K. K.; Simes, G. A Pilot-Scale Demonstration of a Stripping Process for Removal and Recovery of Volatile Organic Compounds. *Environ. Prog.* **2001**, *20* (1), 27–35.

(8) Le Cloirec, P. Adsorption onto Activated Carbon Fiber Cloth and Electrothermal Desorption of Volatile Organic Compound (VOCs): A Specific Review. *Chinese J. Chem. Eng.* **2012**, *20* (3), 461–468 DOI: 10.1016/S1004-9541(11)60207-3.

(9) Montes, M.; Daugulis, A. J.; Veiga, M. C.; Kennes, C. Characterization of absorbent polymers for the removal of volatile hydrophobic pollutants from air. *J. Chem. Technol. Biotechnol.* **2011**, *86* (1), 47–53 DOI: 10.1002/jctb.2517.

(10) Domeño, C.; Rodríguez-Lafuente, A.; Martos, J. M.; Bilbao, R.; Nerín, C. VOC removal and deodorization of effluent gases from an industrial plant by photo-oxidation, chemical oxidation, and ozonization. *Environ. Sci. Technol.* **2010**, *44* (7), 2585–2591 DOI: 10.1021/es902735g.

(11) Sakuma, T.; Hattori, T.; Deshusses, M. A. Comparison of Different Packing Materials for the Biofiltration of Air Toxics. *J. Air Waste Manage. Assoc.* **2006**, *56* (11), 1567–

1575 DOI: 10.1080/10473289.2006.10464564.

(12) Muñoz, R.; Villaverde, S.; Guieysse, B.; Revah, S. Two-phase partitioning bioreactors for treatment of volatile organic compounds. *Biotechnol. Adv.* **2007**, *25* (4), 410–422 DOI: 10.1016/j.biotechadv.2007.03.005.

(13) Quijano, G.; Couvert, A.; Amrane, A.; Darracq, G.; Couriol, C.; Le Cloirec, P.; Paquin, L.; Carrié, D. Absorption and Biodegradation of Hydrophobic Volatile Organic Compounds in Ionic Liquids. *Water, Air, Soil Pollut.* **2013**, *224* (5), 1528, 1–9 DOI: 10.1007/s11270-013-1528-y.

(14) Dumont, E.; Darracq, G.; Couvert, A.; Couriol, C.; Amrane, A.; Thomas, D.; Andrès, Y.; Le Cloirec, P. VOC absorption in a countercurrent packed-bed column using water/silicone oil mixtures: Influence of silicone oil volume fraction. *Chem. Eng. J.* **2011**, *168* (1), 241–248 DOI: 10.1016/j.cej.2010.12.073.

(15) Darracq, G.; Couvert, A.; Couriol, C.; Dumont, E.; Amrane, A.; Le Cloirec, P. Activated Sludge Acclimation for Hydrophobic VOC Removal in a Two-Phase Partitioning Reactor. *Water, Air, Soil Pollut.* **2012**, *223* (6), 3117–3124 DOI: 10.1007/s11270-012-1094-8.

(16) Dumont, E.; Darracq, G.; Couvert, A.; Couriol, C.; Amrane, A.; Thomas, D.; Andrès, Y.; Le Cloirec, P. Hydrophobic VOC absorption in two-phase partitioning bioreactors; influence of silicone oil volume fraction on absorber diameter. *Chem. Eng. Sci.* **2012**, *71*, 146–152 DOI: 10.1016/j.ces.2011.12.017.

(17) Huddleston, J. G.; Visser, A. E.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Rogers, R. D. Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. *Green Chem.* **2001**, *3* (4), 156–164 DOI: 10.1039/b103275p.

(18) Sheldon, R. A. Catalytic reactions in ionic liquids. *Chem. Commun. (Camb).* **2001**, 2399–2407 DOI: 10.1039/b107270f.

(19) Yau, H. M.; Chan, S. J.; George, S. R. D.; Hook, J. M.; Croft, A. K.; Harper, J. B. Ionic liquids: just Molten salts after all? *Molecules* **2009**, *14* (7), 2521–2534 DOI: 10.3390/molecules14072521.

(20) Shkrob, I. A.; Wishart, J. F. Charge trapping in imidazolium ionic liquids. *J. Phys. Chem. B* **2009**, *113* (16), 5582–5592 DOI: 10.1021/jp811495e.

(21) Maiti, A.; Rogers, R. D. A correlation-based predictor for pair-association in ionic liquids. *Phys. Chem. Chem. Phys.* **2011**, *13* (26), 12138–12145 DOI: 10.1039/c1cp21018a.

(22) Coleman, D.; Gathergood, N. Biodegradation studies of ionic liquids. *Chem. Soc. Rev.* **2010**, *39* (2), 600–637 DOI: 10.1039/b817717c.

(23) Chiappe, C.; Pieraccini, D. Ionic liquids: solvent properties and organic reactivity. *J. Phys. Org. Chem.* **2005**, *18* (4), 275–297 DOI: 10.1002/poc.863.

(24) Wilkes, J. S.; Zaworotko, M. J. Air and water stable 1-ethyl-3-methylimidazolium based ionic liquid. *J. Chem. Soc. Chem. Commun.* **1992**, 965–967.

(25) Weingärtner, H. Understanding ionic liquids at the molecular level: facts, problems, and controversies. *Angew. Chem. Int. Ed. Engl.* **2008**, *47* (4), 654–670 DOI: 10.1002/anie.200604951.

(26) Wilkes, J. S. A short history of ionic liquids—from molten salts to neoteric solvents. *Green Chem.* **2002**, *4* (2), 73–80 DOI: 10.1039/b110838g.

(27) Wilkes, J. S.; Levisky, J. A.; Wilson, R. A.; Hussey, C. L. Dialkylimidazolium Chloroaluminate Melts: A New Class of Room-Temperature Ionic Liquids for Electrochemistry, Spectroscopy, and Synthesis. *Inorg. Chem.* **1982**, *237* (1980), 1263–1264.

(28) Welton, T. Room-temperature ionic liquids: solvents for synthesis and catalysis. 2. *Chem. Rev.* **1999**, *99*, 2071–2083 DOI: 10.1021/cr1003248.

(29) Earle, M. J.; Seddon, K. R. Ionic liquids. Green solvents for the future. *Pure Appl. Chem.* **2000**, *72* (7), 1391–1398 DOI: 10.1351/pac200072071391.

(30) Huddleston, J. G.; Willauer, H. D.; Swatloski, R. P.; Visser, A. E.; Rogers, R. D. Room temperature ionic liquids as novel media for " clean " liquid – liquid extraction. *Chem. Commun. (Camb).* **1998**, 1765–1766.

(31) Romero, A.; Santos, A.; Tojo, J.; Rodríguez, A. Toxicity and biodegradability of imidazolium ionic liquids. *J. Hazard. Mater.* **2008**, *151* (1), 268–273 DOI: 10.1016/j.jhazmat.2007.10.079.

(32) Cull, S. G.; Holbrey, J. D.; Vargas-Mora, V.; Seddon, K. R.; Lye, G. J. Room-Temperature Ionic Liquids as Multiphase Bioprocess Operations. *Biotechnol. Bioeng.*

**2000**, *69* (2), 227–233.

(33) Milota, M.; Mosher, P.; Li, K. VOC and HAP removal from dryer exhaust gas by absorption into ionic liquids. *For. Prod. J.* **2007**, *57* (5), 73–77.

(34) Milota, M.; Mosher, P.; Li, K. RTIL absorption of organic emissions from press and dry exhaust. *For. Prod. J.* **2008**, *58* (10366), 97–101.

(35) Quijano, G.; Couvert, A.; Amrane, A. Ionic liquids: applications and future trends in bioreactor technology. *Bioresour. Technol.* **2010**, *101* (23), 8923–8930 DOI: 10.1016/j.biortech.2010.06.161.

(36) Quijano, G.; Couvert, A.; Amrane, A.; Darracq, G.; Couriol, C.; Le Cloirec, P.; Paquin, L.; Carrié, D. Toxicity and biodegradability of ionic liquids: New perspectives towards whole-cell biotechnological applications. *Chem. Eng. J.* **2011**, *174* (1), 27–32 DOI: 10.1016/j.cej.2011.07.055.

(37) Darracq, G.; Couvert, A.; Couriol, C.; Amrane, A.; Thomas, D.; Dumont, E.; Andres, Y.; Le Cloirec, P. Silicone oil: An effective absorbent for the removal of hydrophobic volatile organic compounds. *J. Chem. Technol. Biotechnol.* **2010**, *85* (3), 309–313 DOI: 10.1002/jctb.2331.

(38) Béchora, I.; Couvert, A.; Amrane, A. Absorption and biodegradation of toluene: Optimization of its initial concentration and the biodegradable non-aqueous phase liquid volume fraction. *Int. Biodeterior. Biodegradation* **2015**, *104*, 350–355 DOI: 10.1016/j.ibiod.2015.07.004.

(39) Vuong, M.-D.; Couvert, a.; Couriol, C.; Amrane, a.; Le Cloirec, P.; Renner, C. Determination of the Henry's constant and the mass transfer rate of VOCs in solvents. *Chem. Eng. J.* **2009**, *150* (2-3), 426–430 DOI: 10.1016/j.cej.2009.01.027.

(40) Burrell, A. K.; Del Sesto, R. E.; Baker, S. N.; McCleskey, T. M.; Baker, G. A. The large scale synthesis of pure imidazolium and pyrrolidinium ionic liquids. *Green Chem.* **2007**, *9* (5), 449–454 DOI: 10.1039/b615950h.

(41) Corbera, R.; Sanau, M.; Peris, E. " Cp * Ir ( III )" Complexes with Hemicleaveable Ligands of the Type N-Alkenyl Imidazolin-2-ylidene . Reactivity and Catalytic Properties. *Organometallics* **2007**, *26*, 3492–3498.

(42) González-Álvarez, J.; Blanco-Gomis, D.; Arias-Abrodo, P.; Díaz-Llorente, D.; Ríos-Lombardía, N.; Busto, E.; Gotor-Fernández, V.; Gutiérrez-Álvarez, M. D. Polymeric

imidazolium ionic liquids as valuable stationary phases in gas chromatography: chemical synthesis and full characterization. *Anal. Chim. Acta* **2012**, *721*, 173–181 DOI: 10.1016/j.aca.2012.01.047.

(43) Wang, S.-W.; Liu, W.; Colby, R. H. Counterion Dynamics in Polyurethane-Carboxylate Ionomers with Ionic Liquid Counterions. *Chem. Mater.* **2011**, *23* (7), 1862–1873 DOI: 10.1021/cm103548t.

(44) Fei, Z.; Ang, W. H.; Zhao, D.; Scopelliti, R.; Zvereva, E. E.; Katsyuba, S. A.; Dyson, P. J. Revisiting ether-derivatized imidazolium-based ionic liquids. *J. Phys. Chem. B* **2007**, *111* (34), 10095–10108 DOI: 10.1021/jp073419l.

(45) Branco, L. C.; Rosa, J. N.; Moura Ramos, J. J.; Afonso, C. A. M. Preparation and characterization of new room temperature ionic liquids. *Chem. A Eurpean J. (Weinheim an der Bergstrasse, Ger.* **2002**, *8* (16), 3671–3677 DOI: 10.1002/1521-3765(20020816)8:16<3671::AID-CHEM3671>3.0.CO;2-9.

(46) Carlisle, T. K.; Bara, J. E.; Gabriel, C. J.; Noble, R. D.; Gin, D. L. Interpretation of CO 2 Solubility and Selectivity in Nitrile-Functionalized Room-Temperature Ionic Liquids Using a Group Contribution Approach. *Ind. Eng. Chem. Res.* **2008**, *47*, 7005–7012.

(47) Domańska, U.; Zawadzki, M. Thermodynamic properties of the N-butylisoquinolinium bis(trifluoromethylsulfonyl)imide. *J. Chem. Thermodyn.* **2011**, *43* (6), 989–995 DOI: 10.1016/j.jct.2011.02.006.

(48) Daily, L. A.; Miller, K. M. Correlating Structure with Thermal Properties for a Series of 1-Alkyl-4-methyl-1,2,4-triazolium Ionic Liquids. *J. Org. Chem.* **2013**, *78*, 4196–4201.

(49) Zhao, D.; Fei, Z.; Ang, W. H.; Dyson, P. J. Sulfonium-based Ionic Liquids Incorporating the Allyl Functionality. *Int. J. Mol. Sci.* **2007**, *8*, 304–315.

(50) Baumann, M. D.; Daugulis, A. J.; Jessop, P. G. Phosphonium ionic liquids for degradation of phenol in a two-phase partitioning bioreactor. *Appl. Microbiol. Botechnology* **2005**, *67* (1), 131–137 DOI: 10.1007/s00253-004-1768-2.

(51) Özdemir, İ.; Demir, S.; Gök, Y.; Çetinkaya, E.; Çetinkaya, B. Synthesis of novel palladium–carbene complexes as efficient catalysts for amination of aryl chlorides in ionic liquid. *J. Mol. Catal. A Chem.* **2004**, *222* (1–2), 97–102 DOI: http://dx.doi.org/10.1016/j.molcata.2004.07.024.

(52) Bergmeyer, Hans Ulrich; Gawehn, Karlfried; Williamson, Dermot Hedley; Patricia, L.; Bergmeyer, H. U. *Methods of enzymatic analysis*; Press, A., Ed.; Methods of Enzymatic Analysis; Verlag Chemie: Weinheim; New York; London, 1974.

(53) Witt, S.; Singh, M.; Kalisz, H. M. Structural and kinetic properties of nonglycosylated recombinant Penicillium amagasakiense glucose oxidase expressed in Escherichia coli. *Appl. Environ. Microbiol.* **1998**, *64* (4), 1405–1411.

(54) Stolte, S.; Abdulkarim, S.; Arning, J.; Blomeyer-Nienstedt, A.-K.; Bottin-Weber, U.; Matzke, M.; Ranke, J.; Jastorff, B.; Thöming, J. Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octylimidazolium chloride and electrochemical wastewater treatment of poorly biodegradable compounds. *Green Chem.* **2008**, *10* (2), 214–224 DOI: 10.1039/b713095c.

(55) Garcia, M. T.; Gathergood, N.; Scammells, P. J. Biodegradable ionic liquids : Part II. Effect of the anion and toxicology. *Green Chem.* **2005**, *7* (1), 9–14 DOI: 10.1039/b411922c.

(56) Docherty, K. M.; Kulpa, C. F. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chem.* **2005**, *7* (4), 185–189 DOI: 10.1039/b419172b.

(57) Swatloski, R. P.; Holbrey, J. D.; Memon, S. B.; Caldwell, G. A.; Caldwell, K. A.; Rogers, R. D. Using Caenorhabditis elegans to probe toxicity of 1-alkyl-3-methylimidazolium chloride based ionic liquids. *Chem. Commun. (Camb).* **2004**, 668–669 DOI: 10.1016/S0147-6513(03)00105-2.

(58) Deng, Y.; Besse-Hoggan, P.; Sancelme, M.; Delort, A. M.; Husson, P.; Costa Gomes, M. F. Influence of oxygen functionalities on the environmental impact of imidazolium based ionic liquids. *J. Hazard. Mater.* **2011**, *198*, 165–174 DOI: 10.1016/j.jhazmat.2011.10.024.

(59) Pretti, C.; Chiappe, C.; Pieraccini, D.; Gregori, M.; Abramo, F.; Monni, G.; Intorre, L. Acute toxicity of ionic liquids to the zebrafish (Danio rerio). *Green Chem.* **2006**, *8* (3), 238–240 DOI: 10.1039/b511554j.

(60) Pretti, C.; Chiappe, C.; Baldetti, I.; Brunini, S.; Monni, G.; Intorre, L. Acute toxicity of ionic liquids for three freshwater organisms: Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio. *Ecotoxicol. Environ. Saf.* **2009**, *72* (4), 1170–1176 DOI: 10.1016/j.ecoenv.2008.09.010.

(61) Du, Z.; Zhu, L.; Dong, M.; Wang, J.; Wang, J.; Xie, H.; Liu, T.; Guo, Y. Oxidative stress and genotoxicity of the ionic liquid 1-octyl-3-methylimidazolium bromide in

zebrafish (Danio rerio). *Arch. Environ. Contam. Toxicol.* **2014**, *67* (2), 261–269 DOI: 10.1007/s00244-014-0046-2.

(62) Du, Z.; Zhu, L.; Dong, M.; Wang, J.; Wang, J.; Xie, H.; Zhu, S. Effects of the ionic liquid [Omim]PF6 on antioxidant enzyme systems, ROS and DNA damage in zebrafish (Danio rerio). *Aquat. Toxicol.* **2012**, *124-125*, 91–93 DOI: 10.1016/j.aquatox.2012.08.002.

(63) Dong, M.; Zhu, L.; Zhu, S.; Wang, J.; Wang, J.; Xie, H.; Du, Z. Toxic effects of 1-decyl-3-methylimidazolium bromide ionic liquid on the antioxidant enzyme system and DNA in zebrafish (Danio rerio) livers. *Chemosphere* **2013**, *91* (8), 1107–1112 DOI: 10.1016/j.chemosphere.2013.01.013.

(64) Jacquemin, J.; Husson, P.; Padua, A. A. H.; Majer, V. Density and viscosity of several pure and water-saturated ionic liquids. *Green Chem.* **2006**, *8* (2), 172–180 DOI: 10.1039/b513231b.

(65) Pham, T. P. T.; Cho, C.-W.; Yun, Y.-S. Environmental fate and toxicity of ionic liquids: a review. *Water Res.* **2010**, *44* (2), 352–372 DOI: 10.1016/j.watres.2009.09.030.

(66) Swatloski, R. P.; Holbrey, J. D.; Rogers, R. D. Ionic liquids are not always green: hydrolysis of 1-butyl-3-methylimidazolium hexafluorophosphate. *Green Chem.* **2003**, *5* (4), 361–363 DOI: 10.1039/b304400a.

(67) Ochoa-Herrera, V.; Banihani, Q.; León, G.; Khatri, C.; Field, J. A.; Sierra-Alvarez, R. Toxicity of fluoride to microorganisms in biological wastewater treatment systems. *Water Res.* **2009**, *43* (13), 3177–3186 DOI: 10.1016/j.watres.2009.04.032.

(68) Frade, R. F. M.; Afonso, C. A. M. Impact of ionic liquids in environment and humans: an overview. *Hum. Exp. Toxicol.* **2010**, *29* (12), 1038–1054 DOI: 10.1177/0960327110371259.

(69) Fatemi, M. H.; Izadiyan, P. Cytotoxicity estimation of ionic liquids based on their effective structural features. *Chemosphere* **2011**, *84* (5), 553–563 DOI: 10.1016/j.chemosphere.2011.04.021.

(70) Madria, N.; Arunkumar, T. A.; Nair, N. G.; Vadapalli, A.; Huang, Y.-W.; Jones, S. C.; Reddy, V. P. Ionic liquid electrolytes for lithium batteries: Synthesis, electrochemical, and cytotoxicity studies. *J. Power Sources* **2013**, *234*, 277–284 DOI: 10.1016/j.jpowsour.2013.02.002.

(71) Romero, A.; Santos, A.; Tojo, J.; Rodríguez, A. Toxicity and biodegradability of imidazolium ionic liquids. *J. Hazard. Mater.* **2008**, *151*, 268–273 DOI: 10.1016/j.jhazmat.2007.10.079.

(72) Garcia, M. T.; Gathergood, N.; Scammells, P. J. Biodegradable ionic liquids : Part II. Effect of the anion and toxicology. *Green Chem.* **2005**, *7* (1), 9–14 DOI: 10.1039/b411922c.

(73) Docherty, K. M.; Dixon, J. K.; Kulpa, C. F. Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community. *Biodegradation* **2007**, *18* (4), 481–493 DOI: 10.1007/s10532-006-9081-7.

(74) Stolte, S.; Abdulkarim, S.; Arning, J.; Blomeyer-Nienstedt, A.-K.; Bottin-Weber, U.; Matzke, M.; Ranke, J.; Jastorff, B.; Thöming, J. Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octylimidazolium chloride and electrochemical wastewater treatment of poorly biodegradable compounds. *Green Chem.* **2008**, *10* (2), 214 DOI: 10.1039/b713095c.

(75) Modelli, A.; Sali, A.; Galletti, P.; Samorì, C. Biodegradation of oxygenated and non-oxygenated imidazolium-based ionic liquids in soil. *Chemosphere* **2008**, *73* (8), 1322–1327 DOI: 10.1016/j.chemosphere.2008.07.012.

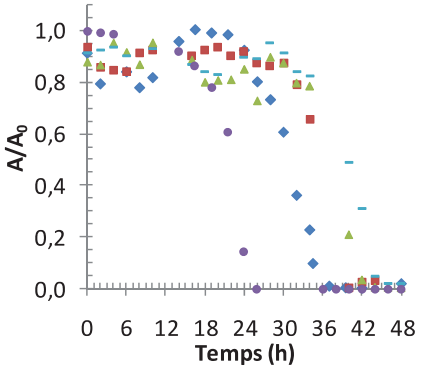



**Figure 1.a Glucose uptake kinetics at 5% (v/v) of IL with the [$NTf_2$] anion; (◆) [Bmim][$NTf_2$], (■) [Butenylmim][$NTf_2$], (▲) [ipentmim][$NTf_2$], (●) [nprmim][$NTf_2$] and (●) control without IL.**

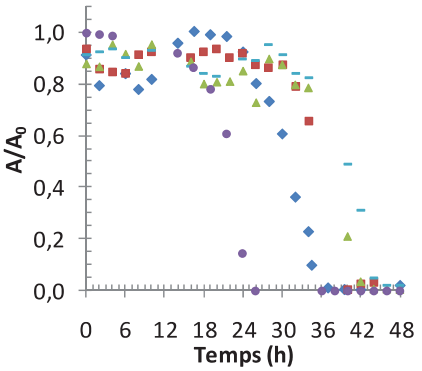



**Figure 1.b Glucose uptake kinetics at 5% (v/v) of IL with the [$NTf_2$] anion; (◆) [OctIq][$NTf_2$], (■) [$C_{10}$Iq][$NTf_2$], (▲) [MeOEIq][$NTf_2$], (●) [EtOEIq][$NTf_2$] and (●) control without IL.**

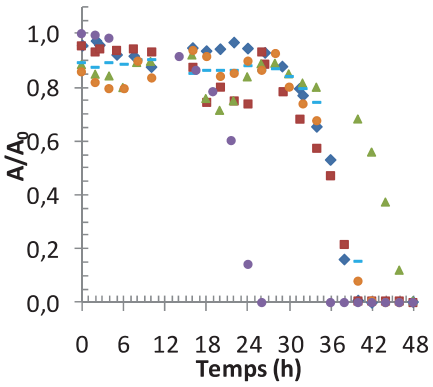



**Figure 1.c Glucose uptake kinetics at 5% (v/v) of IL with the [$NTf_2$] anion; (◆) [MeOmim][$NTf_2$], (■) [EtOEmim][$NTf_2$], (▲) [$MeOC_2OC_2$Mim][$NTf_2$], (▬) [MeOEMMorph][$NTf_2$], (●) [EtOEMMorph][$NTf_2$] and (●) control without IL.**

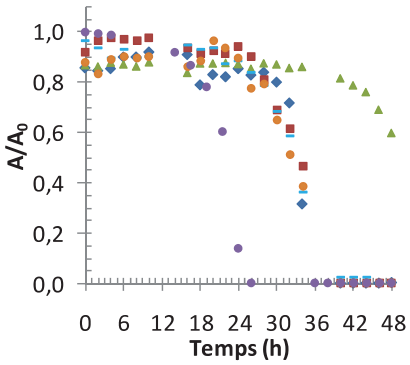



**Figure 1.d Glucose uptake kinetics at 5% (v/v) of IL with the [$NTf_2$] anion; (◆) [$CNC_3$im][$NTf_2$], (■) [BMPyrr][$NTf_2$], (▲) [BMTriaz][$NTf_2$], (▬) [$CF_2$CFEMTriaz][$NTf_2$] (●) [Allyl$Et_2$S][$NTf_2$] and (●) control without IL.**

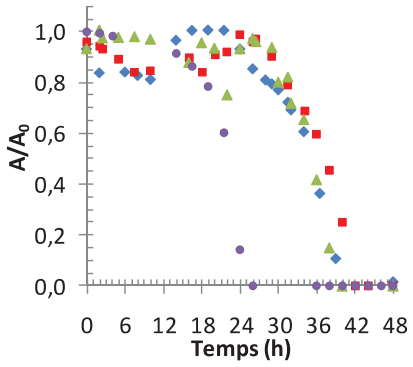



**Figure 1.e Glucose uptake kinetics at 5% (v/v) of IL with the [$PF_6$] anion; (♦) [Bmim][$PF_6$], (■) [ipentmim][$PF_6$], (▲) [Butenylmim][$PF_6$], and (●) control without IL.**

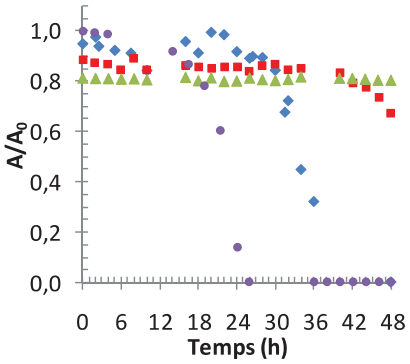



**Figure 1.f Glucose uptake kinetics at 5% (v/v) of IL with the [NfO] anion; (♦) [Bmim][NfO], (■) [Bunetylmim][NfO], (▲) [MeOEmim][NfO], and (●) control without IL.**

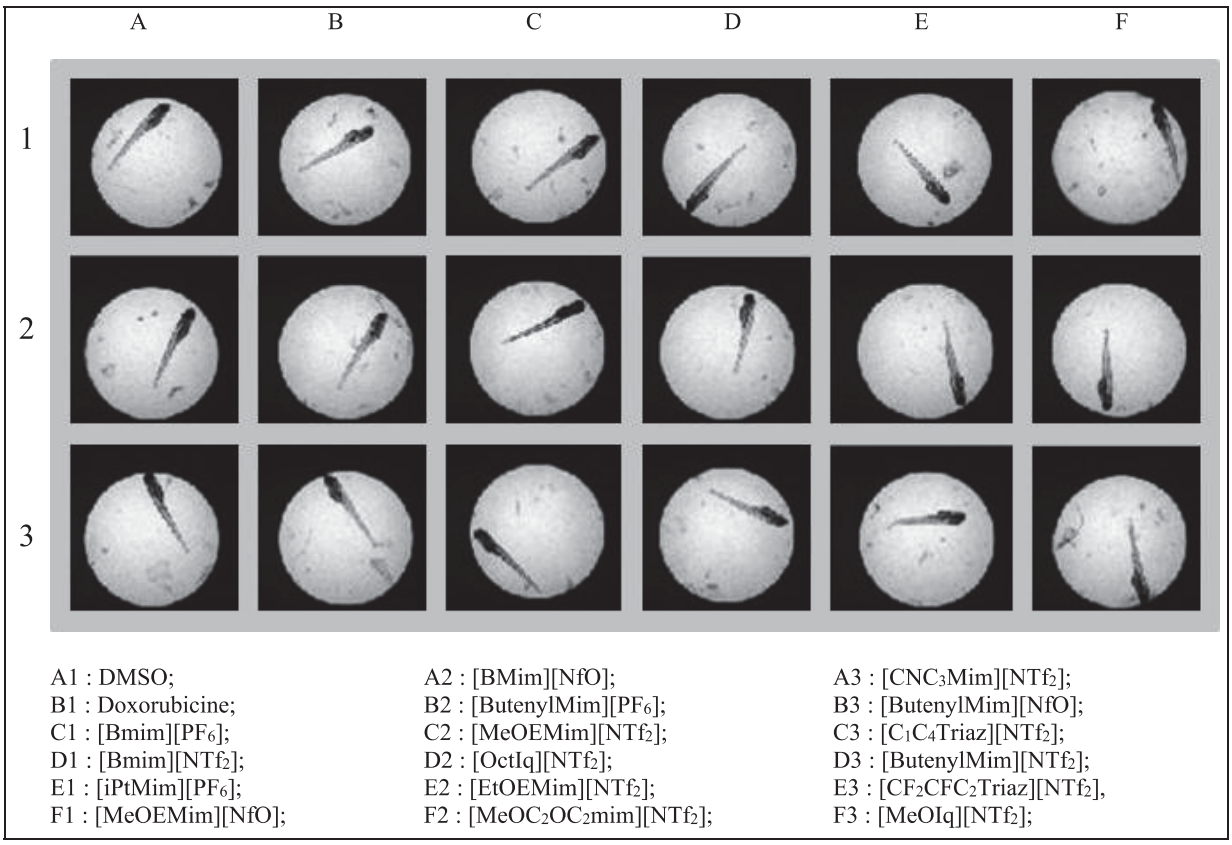



**Figure 2. Zebrafish Pictures after 72h: Doxorubicine (0.5µM); DMSO (0.25µM) and IL (100µM).**

Table 1. Partition coefficients for toluene in several ILs.

| VOC | Liquid Phase | K ($Pa.m^3.mol^{-1}$) | Reference |
|---|---|---|---|
| Toluene | Water | 676.75 | [37] |
| | [nPrmim][$NTf_2$] | 0.73 | Art diffusion |
| | [iPentmim][$NTf_2$] | 0.63 | Art diffusion |
| | [OctIq][$NTf_2$] | 0.5 | Art diffusion |
| | [Allyl$Et_2$S][$NTf_2$] | 0.88 | Art diffusion |
| | Silicone oil (0.0049 kg m s ) | 2.5 | [37] |
| | [Bmim][NTf2] | 1.51 | [37] |
| | [Bmim][PF6] | 2.38 | [37] |
| | Di-(2-ethyl)hexyladipate. | 1.0 | [38] |
| | n-Hexadecane | 1.6 | [39] |

**Table 2 Cytotoxicity References of IC50 in µM for cytotoxicity assays.**

| Compound | Huh7 | Caco2 | MDA | HCT116 | PC3 | NCI | HaCat | Fibroblast |
|---|---|---|---|---|---|---|---|---|
| Diméthylsulfoxide | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Roscovitine | 8 | 8 | 14 | 6 | 7 | 22 | 13 | 3 |
| Doxorubicine | 0.02 | 0.04 | 0.01 | 0.04 | 0.05 | 0.03 | 0.03 | 0.01 |
| Taxol | 0.005 | 0.02 | 0.002 | <0.001 | <0.001 | 0.005 | 0.002 | >0.25 |

**Table 3. Variation of the acclimation time and the total glucose consumption time during toxicity test against activated sludge.**

| Ionic Liquid | Acclimation time (h) | Total glucose consumption time (h) |
|---|---|---|
| Control | 14h | 12h |
| [Bmim][$NTf_2$] | 24h | 10h |
| [Bmim][$PF_6$] | 24h | 16h |
| [Bmim][NfO] | 24h | 14h |
| [nPrmim][$NTf_2$] | 32h | 12h |
| [iPtmim][$PF_6$] | 26h | 18h |
| [iPtmim][$NTf_2$] | 32h | 10h |
| [Butenylmim][$PF_6$] | 26h | 14h |
| [Butenylmim][$NTf_2$] | 24h | 16h |
| [Butenylmim][NfO] | 30h | 18h (-23%) |
| [MeOEmim][$NTf_2$] | 24h | 16h |
| [EtOEmim][$NTf_2$] | 26h | 14h |
| [$MeOC_2OC_2$Mim][$NTf_2$] | 28h | 16h |
| [MeOEmim][NfO] | Total inhibition | Total inhibition |
| [OctIq][$NTf_2$] | 20h | 12h |
| [$C_{10}$Iq][$NTf_2$] | 16h | 12h |
| [MeOEIq][$NTf_2$] | 26h | 16h |
| [EtOEIq][$NTf_2$] | 26h | 16h |
| [$CNC_3$mim][$NTf_2$] | 24h | 14h |
| [BMTriaz][$NTf_2$] | 28h | 20h (-32%) |
| [$CF_3CF_2$BTriaz][$NTf_2$] | 24h | 16h |
| [BMPyrr][$NTf_2$] | 24h | 16h |
| [Allyl$Et_2$S][$NTf_2$] | 26h | 14h |
| [MeOEMMorph][$NTf_2$] | 28h | 14h |
| [EtOEMMorph][$NTf_2$] | 24h | 16h |

**Table 4. Fluoride Releasing (mg.L$^{-1}$) in contact with water (at 25% vol.) in mg.L$^{-1}$ after 30 minutes, 5 days, 19 days, 1 month, 2 months and 3 months under agitation at room temperature.**

| ILs \ Days | 0 | 5 | 19 | 30 | 45 | 60 | 90 |
|---|---|---|---|---|---|---|---|
| [Bmim][$PF_6$] | 0 | 0 | 0 | 0 | 0 | 0.101 | -0.303 |
| [Bmim$NTf_2$] | 0 | 0 | 0 | 0 | 0 | -2.424 | 0 |
| [Butenylmim][$PF_6$] | 0 | 0 | 0 | 0 | ND | 0 | -1.919 |
| [Butenylmim][NfO] | 0 | 0 | 0 | 0 | ND | 0 | 0 |
| [iPtmim][$PF_6$] | 0 | 0 | 0 | 0 | 8.585 | 3.838 | -3.03 |
| [MeOEmim][$NTf_2$] | 0 | 0 | -2.727 | 0 | -1.111 | -0.202 | 0 |
| [MeOEMim][NfO] | ND | 0 | 0 | 0 | 2.222 | 0 | -0.606 |
| [EtOEmim][$NTf_2$] | 0 | 0 | 0 | 0 | -5.05 | ND | 0 |
| [$MeOC_2OC_2$Mim][$NTf_2$] | 0 | 0 | 0 | 0 | -2.828 | 0 | 0 |
| [OctIq][$NTf_2$] | 0 | 0 | 0 | 0 | -1.818 | -3.232 | 0 |
| [MeOEIq][$NTf_2$] | 0 | 0 | 0 | -1.616 | -1.414 | -4.141 | 0 |
| [$CNC_3$mim][$NTf_2$] | 0 | ND | 0 | 0 | 0 | 0 | 0 |
| [HexIq][$NTf_2$] | 0 | 0 | ND | 4.848 | 0 | 0 | 0 |
| [BMPyrr][$NTf_2$] | ND | 0 | ND | 0 | 2.222 | ND | 0 |
| [MeOEmim][NfO] | ND | 0 | 0 | 0 | 2.222 | 0 | -0.606 |
| [Butenylmim][NfO] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [BMTriaz][$NTf_2$] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [MeOEMMorph][$NTf_2$] | 0 | 0 | ND | 0 | 0 | 0 | 0 |
| [Butenylmim][$NTf_2$] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [$CF_2$CFEBTriaz][$NTf_2$] | 218.867 | 491.365 | ND | 700.435 | ND | 913.04 | 1150.087 |
| [AllylEt$_2$S][$NTf_2$] | -2.828 | 0 | 0 | 0 | 0 | 0 | 0 |
| [iPtmim][$NTf_2$] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [nPrmimN$Tf_2$] | ND | 0 | 0 | 0 | 0 | 0 | 0 |
| [$C_{10}$Iq][$NTf_2$] | ND | 0 | 0 | 0 | 0 | 0 | 0 |
| [EtOEMMorph][$NTf_2$] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [EtOEIq][$NTf_2$] | ND | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 5. Cytotoxicity Results $IC_{50}$ (µM) for cytotoxic ILs.**

| Compound | Huh7 | Caco2 | MDA | HCT116 | PC3 | NCI | HaCat | Fibroblast |
|---|---|---|---|---|---|---|---|---|
| [OctIq][$NTf_2$] | 5 | 8 | 16 | 4 | 6 | 4 | 4 | 7 |
| [OctIq][NfO] | 1 | 9 | 31 | 6 | 11 | 6 | 5 | >25 |
| [HexIq][$NTf_2$] | 4 | 12 | >25 | 5 | >25 | 2 | 13 | >25 |
| [$C_{10}$Iq][$NTf_2$] | 2 | 2 | 10 | 1 | 2 | 3 | 1 | >25 |

**Table 6. Biodegradability tests based on $BOD_{28}$ experiments.**

| Ionic Liquid | $BOD_{28}$ (mg $L^{-1}$ $O_2$) | COD (mg $L^{-1}$ $O_2$) | % Biodegradation |
|---|---|---|---|
| Control (endogenous breath) | 25.8±8.9 | - | - |
| Control (glutamic acid+glucose) | 276.3±35.9 | - | - |
| [Bmim][$Ntf_2$] | 8.4 | 312.0 | 0 |
| [Bmim][$PF_6$] | 8.6 | 311.0 | 0 |
| [Bmim][NfO] | 14.1 | 298.0 | 0 |
| [nPrmim][$Ntf_2$] | 11.3 | 263.0 | 0 |
| [iPtmim][$PF_6$] | 5.6 | 345.0 | 0 |
| [iPtmim][$Ntf_2$] | 8.4 | 332.0 | 0 |
| [Butenylmim][$PF_6$] | 7.1 | 322.0 | 0 |
| [Butenylmim][$Ntf_2$] | 5.6 | 324.0 | 0 |
| [Butenylmim][NfO] | 1.4 | 368.0 | 0 |
| [MeOEmim][$Ntf_2$] | 15.5 | 275.8 | 0 |
| [EtOEmim][$Ntf_2$] | 11.3 | 261.0 | 0 |
| [$MeOC_2OC_2$Mim][$NTf_2$] | 11.3 | 277.0 | 0 |
| [MeOEmim][NfO] | 2.8 | 312.0 | 0 |
| [OctIq][$NTf_2$] | 18.3 | 176.0 | 0 |
| [$C_{10}$Iq][$NTf_2$] | 9.9 | 80.0 | 0 |
| [MeOEIq][$Ntf_2$] | 19.7 | 362.0 | 0 |
| [EtOEIq][$Ntf_2$] | 11.3 | 390.0 | 0 |
| [$CNC_3$mim][$Ntf_2$] | 4.2 | 151.4 | 0 |
| [BMTriaz][$Ntf_2$] | 1.4 | 111.0 | 0 |
| [$CF_3CF_2$BTriaz][$Ntf_2$] | 8.4 | 167.8 | 0 |
| [BMPyrr][$Ntf_2$] | 7.0 | 189.7 | 0 |
| [Allyl$Et_2$S][$Ntf_2$] | 9.9 | 172.6 | 0 |
| [MeOEMMorph][$Ntf_2$] | 12.7 | 127.4 | 0 |
| [EtOEMMorph][$NTf_2$] | 11.3 | 159.6 | 0 |